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CLINICAL INVESTIGATION

Increased expression of a brain/embryo-type myosin heavy chain isoform (MIIB2) in mesangial proliferative glomerulonephritis

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Increased expression of a brain/embryo-type myosin heavy chain isoform (MIIB2) in mesangial proliferative glomerulonephritis. Proliferation of mesangial cells is frequently found in glomerulonephritis, such as IgA glomerulonephritis. Recent reports suggest that a brain/embryo-type myosin isoform (MIIB2) is involved in cell proliferation. We have studied the expression of MIIB2 in renal biopsy samples from patients with various renal diseases and in the renal tissues from the rat model of mesangial proliferative glomerulonephritis induced with anti-Thy 1.1 antibody. Immunohistochemical analysis of the biopsy samples using an anti-brain-type myosin heavy chain-specific monoclonal antibody (HBM1) indicated that 92% of the samples from patients with IgA glomerulonephritis contained a significant population of mesangial cells that reacted with the antibody. Most of the samples from patients with other types of proliferative glomerular diseases also contained HBM1-reactive mesangial cells, while none of the samples from patients with non-proliferative glomerular diseases contained a significant population of HBM1-reactive mesangial cells. The expression of a brain/embryo-type myosin heavy chain isoform (MIIB2) in the mesangial cells began at five days after injection of anti-Thy 1.1 antibody and peaked at the tenth day. On the other hand, the expression of the proliferating cell nuclear antigen in the mesangial cells was induced at two days after injection of anti-Thy 1.1 antibody and was maximal at the fourth day. These results indicate that the expression of the MIIB2 isoform by mesangial cells is accelerated in proliferative glomerulonephritis and suggest that the myosin isoform is involved in the phenotypic transformations of the glomerular tissues rather than in the cell proliferation.

Mesangial cells are recognized as myofibroblasts because of several features shared with vascular smooth muscle cells and fibroblasts [1]. It has been shown that post-confluent rat mesangial cells in primary culture express three distinct myosin heavy chain (MHC) isoforms, a major MHC of 196 kDa and two minor MHCs of 204 and 200 kDa [2], as in cultured rat aortic smooth muscle cells [3, 4]. These 196, 204 and 200 kDa MHCs were shown to be the nonmuscle-type isoform (MIIA) and the smooth muscle-type isoforms, SM1 and SM2, respectively [2]. It has also been shown that the levels of the smooth muscle-type MHC isoforms relative to that of the nonmuscle isoform further decrease in long phase cultures of the mesangial cells [1], as observed for the cultured aortic smooth muscle cells [3, 4].

Brain tissues contain at least three nonmuscle-type MHC isoforms, two brain-type MHCs of 200 and 198 kDa (brain-specific-type and brain/embryo-type MHCs, MIIB1 or NM1 and MIIB2 or NM2, respectively) and a non-muscle-type MHC of 196 kDa (MIIA or NM3) [5–7]. The MIIB1 is likely to be an alternatively spliced product of the MIIB2 gene with an inserted sequence of about 2 kDa in the head region [8, 9]. Whereas the MIIB1 isoform is expressed almost exclusively in the brain, especially in the cerebellum, the MIIB2 (brain/embryo-type) isoform is also expressed in small amounts in other limited tissues such as kidney and adrenal, and in many fetal and neonatal tissues [5–7, 10, 11]. Recent studies also indicate that the neointimas of atherosclerotic arteries contain an increased concentration of the MIIB2 isoform [12, 13]. These results appear to suggest that expression of the MIIB2 isoform is accelerated in actively proliferating tissues and that the isoform is involved in the proliferation and/or phenotypic transformations of the tissues.

One of the central features of many glomerular diseases is the proliferation of the mesangial cells [14–16]. Concomitantly with the cellular proliferation, these cells are also known to undergo a variety of phenotypic changes as characterized by the changes in cell morphology, expression of α-smooth muscle actin, increased secretion of extracellular matrix components and increased release of proteolytic enzymes and inflammatory mediators [14–18]. While at least three distinct growth factors, PDGF, bFGF and TGF-β, have been shown to be involved in the proliferation and phenotypic transformations [16], the mechanisms that underly the changes are not elucidated. In the present study, to examine whether the proliferation and transformation of the mesangial cells involve the changes in the level of the brain/embryo-type myosin isoform, we have studied the expression of the brain/embryonic myosin isoform MIIB2 in the renal biopsy specimens of patients with a variety of renal diseases and in the renal tissues from the rat model of mesangial proliferative glomerulonephritis (GN) induced with anti-Thy 1.1 antibody [19–21].

Methods

Patients and tissue samples

Tissue samples were obtained by percutaneous renal biopsy of 36 patients with renal diseases with ages of 14 to 75 in Wakayama

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Table 1. Clinical data on patients with renal diseases and expression of brain-type MHC in mesangial cells of the biopsy specimens

Age	Sex	Onset	Macrohematuria	Hypertension	Consistent proteinuria	Histology	Clinical diagnosis	HBM1[a]	Mesangial cell hypercellularity[b]
45	M	42	−	−	+	IgA GN	CGN	+	+
36	F	35	+	−	+	IgA GN	CGN	+	+
45	F	43	+	−	+	IgA GN	CGN	+	2+
14	M	14	+	−	−	IgA GN	CGN	+	+
51	F	49	−	−	+	IgA GN	CGN	+	+
45	M	45	−	−	+	IgA GN	CGN	+	+
47	F	26	−	+	+	IgA GN	CGN	+	+
71	M	51	−	+	+	IgA GN	CGN	+	−
44	F	44	+	−	+	IgA GN	CGN	+	+
46	M	42	−	−	+	IgA GN	CGN	+	+
29	M	27	−	−	+	IgA GN	CGN	+	+
21	M	16	−	−	+	IgA GN	CGN	+	+
29	F	21	−	−	+	IgA GN	CGN	−	+
23	F	23	−	−	−	focal proliferative GN	Sjögren syndrome	±	+
21	M	21	−	−	−	acute tubular necrosis	acute renal failure	−	−
28	F	15	−	−	−	MGA	CGN	−	−
60	M	40	−	−	+	FGS	CGN	−	−
15	F	8	−	−	+	lupus nephritis	SLE	+	+
56	F	56	−	−	+	renal amyloidosis	nephrotic syndrome	−	−
21	F	15	−	−	+	MGN	CGN	+	+
37	F	32	−	+	+	lupus nephritis	SLE, nephrotic syndrome	±	+
75	M	74	−	−	+	renal amyloidosis	Amyloidosis	−	−
69	M	68	−	+	+	MGN	nephrotic syndrome	−	−
54	M	54	−	−	+	MGN	nephrotic syndrome	+	+
59	F	58	−	−	+	MGN	CGN	+	+
59	F	45	−	+	+	FGO	CGN	−	−
26	M	26	−	−	+	MGA	nephrotic syndrome	−	−
33	F	26	−	−	+	MGA	CGN	−	−
45	F	43	−	−	+	focal proliferative GN	CGN	±	+
25	F	17	−	−	−	MGA	CGN	−	−
65	F	60	−	−	+	MGN	CGN	−	−
40	F	40	−	−	+	MGA	nephrotic syndrome	−	−
55	M	42	−	−	+	MGN	CGN	−	−
22	F	21	+	−	−	MGA	CGN	−	−
45	F	40	−	−	+	MGN	CGN	−	−
28	F	27	−	−	+	MGA	nephrotic syndrome	−	−

Abbreviations are: IgA GN, IgA glomerulonephritis; MGA, minor glomerular abnormalities; FGS, focal glomerular sclerosis; MGN, membranous glomerulonephritis; FGO, focal glomerular obsolescence; CGN, chronic glomerulonephritis; SLE, systemic lupus erythematosus.

[a] The intensity of immunofluorescence was graded as negative (−), trace (±), and positive (+)

[b] The grading of mesangial cell hypercellularity was divided into four grades as negative (−), mild (+), moderate (2+) and severe (3+)

Medical College Hospital and the affilated hospitals and employed for histological, immunohistochemical, and immunofluorescence studies. The 13 patients were diagnosed as IgA glomerulonephritis (GN) and the other 23 patients were affected by other types of renal diseases, as listed in Table 1. Renal tissues were also obtained from patients with renal carcinoma ($N = 3$) or renovascular hypertension ($N = 1$) by surgical operation, and the peripheral normal tissues were employed as the control.

Induction of nephritis in rats by anti-Thy 1.1 antibody

Female Wistar rats (7 weeks old) were injected intravenously with 100 μl of ascites fluid containing anti-rat Thy 1.1 monoclonal antibody (MRC OX-7) (CEDARLANE, Canada) [19–21]. The kidneys were removed at multiple time points ranging from one day to four weeks each time from the four rats. These kidneys were donated for the histological and immunohistological studies and for the preparation of glomeruli [22].

Monoclonal anti-myosin antibodies

BBM4 (IgM), which reacted efficiently with every type of smooth muscle and nonmuscle MHC isoforms but not with skeletal or cardiac MHC isoform, was obtained by immunizing mice with purified bovine cerebrum myosin as described previously [5]. HBM1 (IgG1) and HBM3 (IgG1) which were specific for brain-type and nonmuscle-type MHC isoforms, respectively, were produced by immunizing mice with purified human cerebrum myosin as described previously [6]. Monoclonal anti-SM1 and anti-SM2 antibodies were generous gifts from Yamasa Shoyu Co. [12, 13].

Histological studies

The biopsy specimens were cut into 4 μ thin sections and stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), periodic acid-methenamine silver (PAM) or Masson-trichrome to characterize the tissues. The extent of mesangial cell hypercellularity was graded as follows; negative (−), mild (+), moderate (2+) and severe (3+).

Immunofluorescence studies

Renal tissue samples were quickly frozen in dry ice and acetone, embedded in OCT (Miles Scientific) and then sectioned at a thickness of 2 μ by a cryostat. The sections were rinsed three times

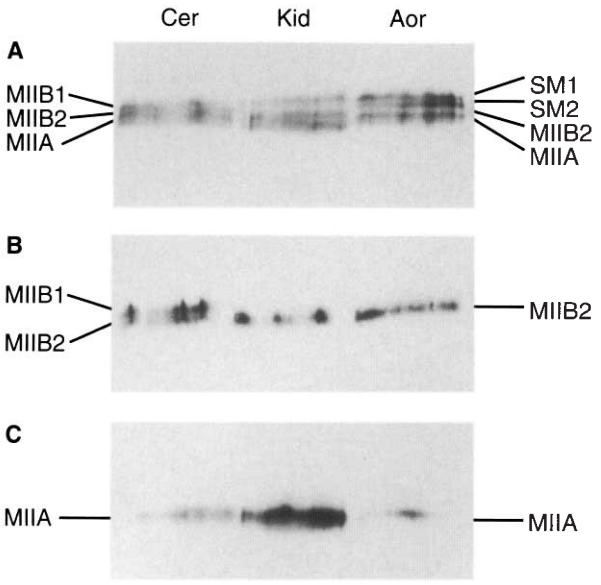

Fig. 1. *Immunoblot analysis of MHC isoforms in crude extracts from human cerebrum, kidney and aorta.* High-salt/Mg-ATP extracts (30 μg protein/lane) from human cerebrum (Cer), kidney (Kid) and aorta (Ao) were subjected to SDS-PAGE and immunoblotting with (**A**) anti-smooth muscle and nonmuscle MHC antibody BBM4, (**B**) anti-brain-type MHC antibody HBM1 or (**C**) anti-nonmuscle MHC antibody. Abbreviations are: MIIB1, brain-specific-type MHC; MIIB2, brain/embryo-type MHC; MIIA, nonmuscle MHC; SM1, smooth muscle MHC-1; SM2, smooth muscle MHC-2.

in 10 mM phosphate-buffered saline (pH 7.4) (PBS) each time for 15 minutes, incubated with either FITC-labeled polyclonal antibodies against human IgA, IgG, IgM, fibrinogen (Cappel), C1q, or C3c (DAKO) for one hour at room temperature, rinsed three times in PBS each time for 30 minutes, and then mounted in glycerin. For analysis of brain-type myosin, the thin sections were incubated with HBM1 (10 μg/ml) for four hours at 4°C, washed three times in PBS each time for 10 minutes and then incubated for one hour with fluorescein-conjugated goat anti-mouse IgG (Cappel) which was preadsorbed with normal human serum. The intensity of immunofluorescence was graded as negative (−), trace (±) or positive (+).

Immunohistochemical staining

Frozen samples were cut into a thickness of 2 μ and placed on silanized slides. The samples were incubated with 3% hydrogen peroxide for five minutes at room temperature to inactivate endogenous peroxidase activity and then incubated with 3% normal rabbit serum to block the nonspecific protein binding sites. The sample sections were incubated with HBM1(2 μg/ml) for one hour at room temperature in a moist chamber. The sections were incubated with a biotin-conjugated rabbit anti-mouse IgG, IgM and IgA (NICHIREI) as the second antibody and then with streptoavidin-horseradish peroxidase conjugate (NICHIREI). For visualizing the reaction products, the sections were incubated with 3-amino-9-ethylcarbazole, a substrate for the peroxidase. Proliferating cell nuclear antigen (PCNA) was stained with a monoclonal antibody (PC 10) specific for PCNA (DAKO) [23, 24]. Counter-staining of the sections was performed with hematoxylin. The index of PCNA expression was determined by counting the numbers of the total and PCNA-positive nuclei in more than ten glomeruli in photomicrographs. Macrophage infiltration into glomeruli was analyzed by immunohistochemical staining CD68

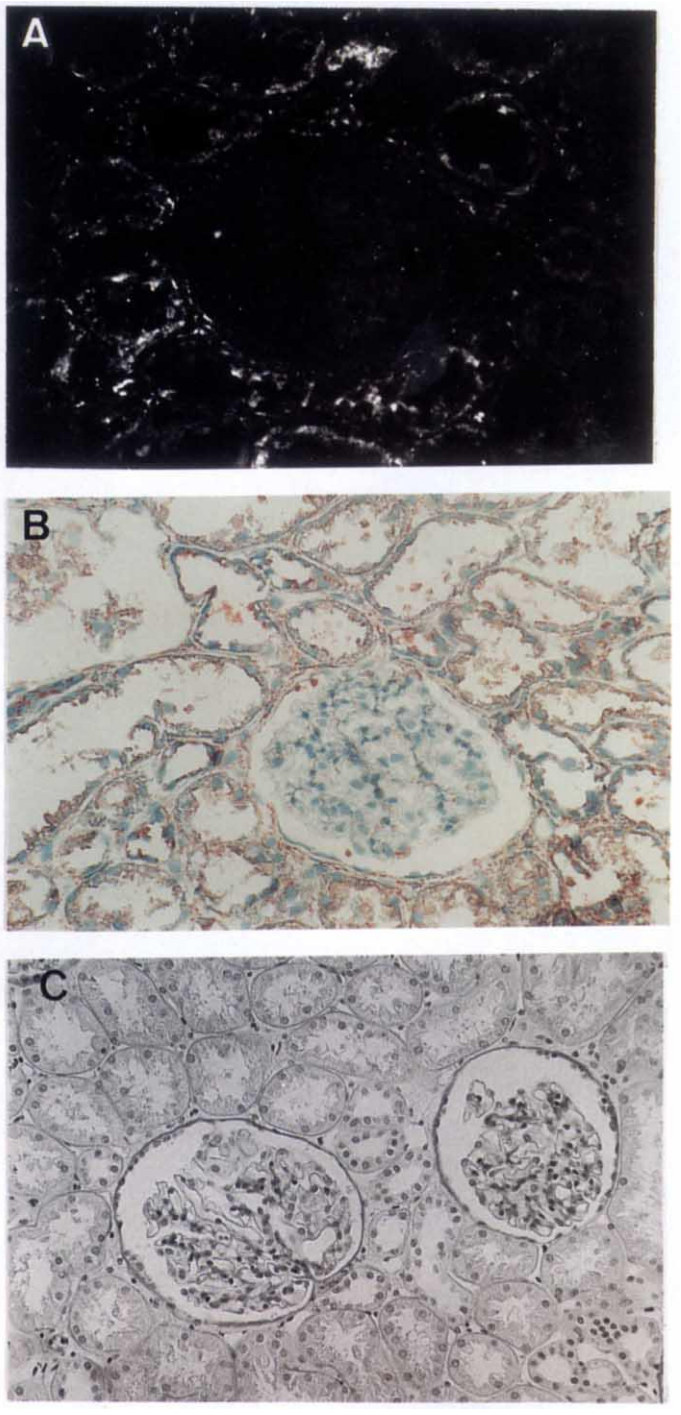

Fig. 2. *Immunofluorescence and histochemical examination of normal tissue specimens from control kidney.* (**A**) Immunofluorescence staining with HBM1 indicates that the tissue contains a small level of brain-type MHC in the tubulointerstitial areas but not appreciably in the mesangial areas (×200). (**B**) Immunohistochemical staining with HBM1 indicates that the immunostains are associated almost only with the tubular areas (×100). (**C**) Examination by light microscopy indicates that the glomeruli are morphologically normal (PAS stain, × 100).

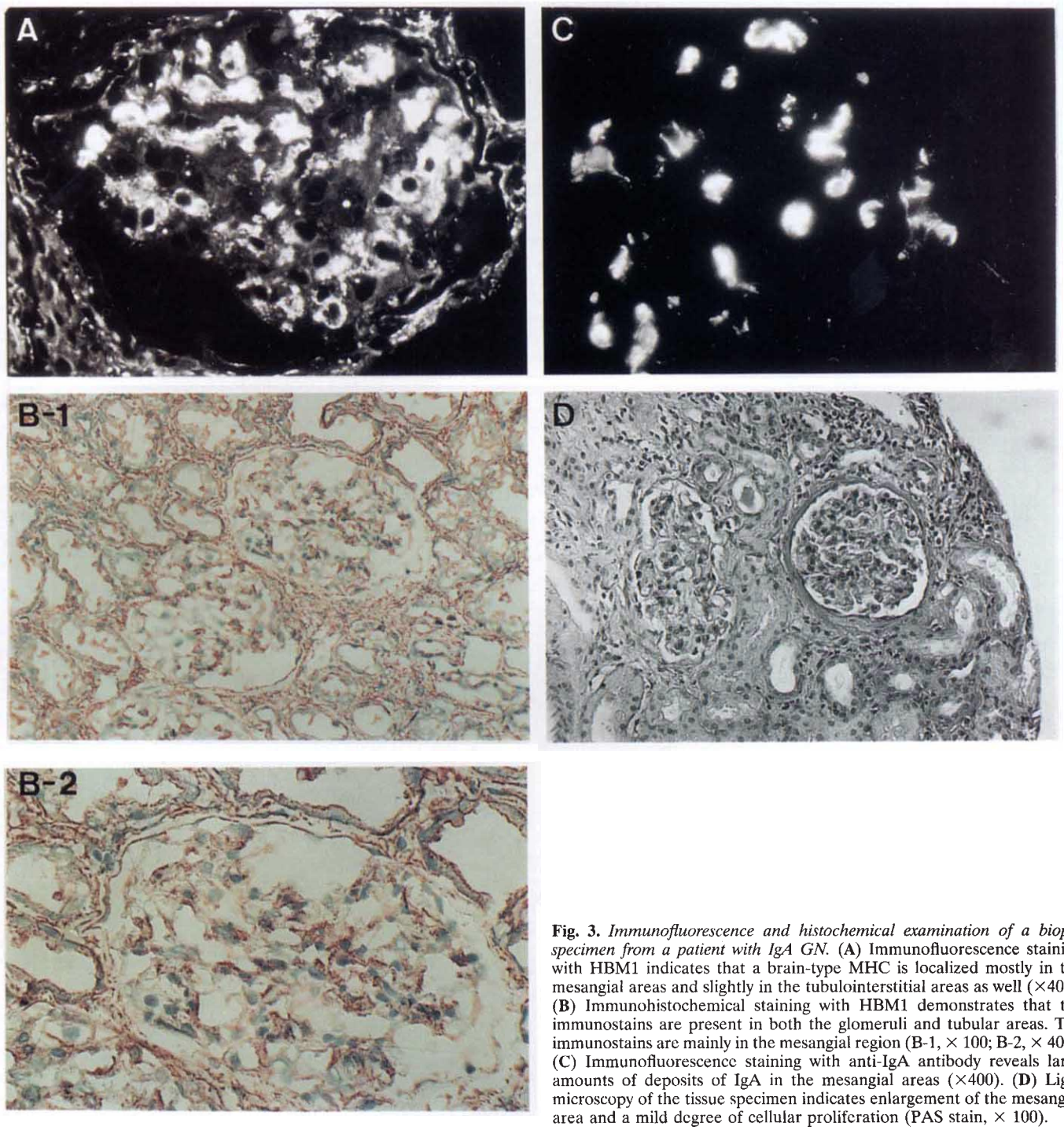

Fig. 3. *Immunofluorescence and histochemical examination of a biopsy specimen from a patient with IgA GN.* (**A**) Immunofluorescence staining with HBM1 indicates that a brain-type MHC is localized mostly in the mesangial areas and slightly in the tubulointerstitial areas as well (×400). (**B**) Immunohistochemical staining with HBM1 demonstrates that the immunostains are present in both the glomeruli and tubular areas. The immunostains are mainly in the mesangial region (B-1, × 100; B-2, × 400). (**C**) Immunofluorescence staining with anti-IgA antibody reveals large amounts of deposits of IgA in the mesangial areas (×400). (**D**) Light microscopy of the tissue specimen indicates enlargement of the mesangial area and a mild degree of cellular proliferation (PAS stain, × 100).

(DAKO) [25]. The renal tissues of rats were incubated with biotinylated HBM1 and then with the streptoavidin-horseradish peroxidase conjugate.

Extraction of myosin from tissues

Tissue samples from human kidney, brain and aorta and the rat glomerular samples were homogenized in a large volume of a low-salt solution containing 10 mM imidazole-HCl (pH 7.0), 5 mM ethylene glycol bis(β-aminoethylether)-N, N, N′, N′-tetraacetic acid (EGTA), 5 mM EDTA, 5 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine-HCl and each 0.1 mg/ml of pepstatin A, leupeptin, aprotinin, and soybean trypsin inhibitor [5, 6]. The homogenates were centrifuged for 10 minutes at 15,000 × g and the supernatants were discarded. The pellets were extracted with a 25 mM Tris-HCl (pH 7.5) buffer containing 0.3 M NaCl, 10 mM $MgCl_2$, 10 mM ATP, 1 mM EGTA, 5 mM 2-mercaptoethanol and the protease inhibitors, and the extracts (high-salt/Mg-ATP extracts) were donated for MHC

analysis. Human aorta was homogenized with the low-salt solution containing 1% Triton X-100.

Immunoblotting

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out as described by Laemmli [26]. The polypeptides separated in the gels were transferred onto polyvinylidene difluoride (PVDF) membrane sheets (Millipore) and then blotted with monoclonal anti-myosin antibodies. The primary antibodies bound to the membrane sheets were detected with biotin-conjugated anti-mouse IgG or IgM (CALTAG) followed by streptoavidin-horseradish peroxidase conjugate (Amersham) and 4-chloro-1-naphtol [5, 6].

Enzyme-linked immunosorbent assay (ELISA)

HBM1-coated 96-well plates were prepared by incubating overnight at 4°C with 100 μl/well of HBM1 solution (10 μg/ml in 10 mM phosphate buffered saline, pH 7.4) and blocked with 0.3% gelatin in 10 mM Tris-HCl, pH 7.4, and 0.15 M NaCl. Crude myosin extracts from rat glomeruli (10 μg protein in 100 μl of 10 mM Tris-HCl, pH 7.4, containing 0.5 M NaCl, 0.3% gelatin and 0.05% Tween-20) were incubated on the HMB1-coated plates for one hour at room temperature. The myosins bound to HBM1 were detected by using biotin-conjugated BBM4 as a detector antibody followed by avidin-horseradish peroxidase and o-phenylenediamine as reagents for color development. The bound myosins were calibrated with purified human brain myosin as a standard.

Statistical analysis

The relationship between the degree of hypercellularity of the mesangial cells and the intensity of staining by HBM1 were assessed by a chi-squared test. Significance was taken at the level of $P < 0.05$.

Results

MHC isoforms in normal renal tissues

When crude extracts from human cerebrum, kidney and aorta were subjected to SDS-PAGE and immunoblotting with a monoclonal antibody BBM4, which was able to react with various types of smooth muscle and nonmuscle MHCs [5], both kidney and aortic extracts gave four bands at the positions of 204, 200, 198 and 196 kDa, while the cerebral extracts gave three bands at the positions of 200, 198 and 196 kDa (Fig. 1A). When these extracts were blotted with a brain-type MHC-specific monoclonal antibody HBM1, the cerebral extracts gave two bands at the positions of 200 and 198 kDa (MIIB1 and MIIB2, respectively), while both kidney and aortic extracts gave only one band at the position of 198 kDa (MIIB2) (Fig. 1B). The 196 kDa MHC bands in all of the extracts were also recognized by a nonmuscle MHC-specific monoclonal antibody HBM3, and the kidney extract appeared to contain a much higher proportion of this MHC than the extracts from the cerebrum and aorta (Fig. 1C). The 204 kDa MHC bands in the kidney and aortic extracts were also recognized by a monoclonal antibody specific to smooth muscle MHC-1 (SM1) [13], while the 200 kDa MHCs in both extracts reacted with a smooth muscle MHC-2 (SM2)-specific monoclonal antibody [13], while the concentration of this 200 kDa band in the kidney extracts was very low (not shown). These results indicate that normal human renal tissues contain at least four distinct MHC isoforms, one nonmuscle MHC isoform (MIIA), one brain/embryo-type MHC isoform (MIIB2) and two smooth muscle MHC isoforms (SM1 and SM2), although the concentration of SM2 is very low compared to those of the other isoforms. It was also evident that normal kidney did not contain an appreciable concentration of MIIB1, a brain-specific MHC isoform.

Immuno-histochemical analysis of brain-type MHC in renal tissues from patients with renal diseases

When normal renal tissue samples from a patients with carcinoma or renovascular hypertension were stained with HBM1 and fluorescent second antibody, the tissues contained a significant population of weakly fluorescent cells in the tubular areas, while the glomerular areas did not apprear to contain an appreciable population of fluorescent cells (Fig. 2A). Immunohistochemical staining with HBM1 and peroxidase-conjugated second antibody also indicated that only the tubular cells were reactive with HBM1 (Fig. 2B). The glomeruli in the same tissue sample had an normal morphology under the light microscope (Fig. 2C). On the other hand, on immunofluorescence staining with HBM1 of a renal tissue specimen from a patient with IgA GN, the glomerular areas contained strongly fluorescent cells, while the tubular areas contained only weakly fluorescent cells as in the normal tissues (Fig. 3A). Immunohistochemical staining of the specimen with HBM1 indicated that most of the immuno-reactive materials in the glomeruli were associated with the mesangial cells (Fig. 3B). In these tissues, a considerable amount of IgA was deposited in the mesangial areas (Fig. 3C), and the mesangial cells appeared to be enlarged (Fig. 3D). These results suggested that the glomeruli from a patient with IgA GN contain an increased concentration of a brain-type MHC isoform in the mesangial areas, while the concentration of the isoform in the tubular areas was low as in the normal tissues. In order to examine whether the expression of the brain-type MHC isoform is increased in the glomeruli of every patient with IgA GN or not, as well as to examine whether the increased expression of the MHC isoform is specific to IgA GN or not, biopsy specimens from other 35 patients with various types of renal diseases were analyzed by the same immunofluorescence staining method as described above. The renal tissue specimens from the 12 patients (92%) out of the 13 patients with IgA GN were found to be highly reactive with HBM1 (Table 1), and large portions of the fluorescent antibody were located in the glomerular cells. On the other hand, when renal tissue specimens from the other 23 patients with different types of renal diseases were examined, the specimens from the 7 patients (31%) contained a significant population of HBM1-reactive mesangial cells. The specimens from the four patients with apparently enlarged mesangial areas were highly reactive with the antibody, while those from the other three patients with only slightly enlarged mesangial areas were only weakly reactive with the antibody (Table 1). The four patients with lupus nephritis, focal proliferative GN in proliferative renal diseases were accompanied by very mild mesangial cell proliferation, and the renal specimens from these patients were not strongly reactive with HBM-1. None of the specimens from the other 16 patients with non-proliferative renal diseases appeared to contain a significant population of HBM1-reactive mesangial cells in the glomeruli. But mild mesangial cell proliferation occurred exceptionally in three patients out of the

seven patients with membranous nephropathy. The specimens from the three patients were reactive with HBM-1. Thus, the level of a brain-type MHC was high in the mesangial cells of proliferative GN but was low in the glomeruli of non-proliferative renal diseases. A statistical analysis indicated that a significantly positive correlation ($P < 0.05$) existed between the mesangial hypercellularity and the level of HBM1-reactive MHC isoform in the glomeruli. On the other hand, it was found that most of the HBM1-reactive glomeruli did not express a significant level of the proliferative cell nuclear antigen (PCNA), a specific marker for the cell in S-phase of cell division [23, 24] (not shown). It was unlikely, therefore, that the HBM1-positive glomerular cells were in an actively proliferating state, but these cells were likely to be in a post-proliferated. An immunohistochemical analysis of the renal specimens using an antibody against anti-CD68, a specific marker for macrophage [25], revealed that none of the HBM1-reactive glomeruli contain an appreciable level of the antigen, indicating that infiltration of macrophages was not significant (not shown). Furthermore, the expression of the brain-type MHC isoform in mesangial cells did not appear to be affected by other factors such as age, sex, age of onset of the disease, macroscopic hematuria, high blood pressure or proteinuria (Table 1).

Expression of MIIB2 isoform in rat anti-Thy 1.1-induced GN

To examine the relationship between the development of proliferative GN and the expression of the brain-type MHC isoform, experimental GN was induced in rats by injecting anti-Thy 1.1 antibody [19–21], and the time course of the changes in the expression of the brain-type MHC in the renal tissues was analyzed. Histological examination of the renal tissues from the Thy 1.1 injected rats indicated that mesangiolysis took place in as early as one day after injection of the antibody and was then followed by proliferation of the cells (Fig. 4), as reported previously [19–21]. Consistently with the morphological changes, the renal tissues began to express PCNA at two days after injection of the antibody and continued to express increasing quantity of PCNA up to four to five days and then ceased to express the protein thereafter (Fig. 5). Immunohistochemical analysis using HBM1 of the renal tissues indicated that the control tissues did not contain significant number of HBM1-positive cells in the glomeruli (Fig. 6A). The Thy 1.1-injected rats appeared to express the brain-type MHC isoform in the mesangial areas at about five days after injection of the antibody and to continue to express increasing quantity of the isoform up to about the tenth day (Fig. 6B, C). To identify the isoform of MHC expressed in the glomeruli, rather than in the whole renal tissues, as well as to quantify the MHC isoform, the glomeruli were isolated from the rat kidneys, and the extracts were subjected to SDS-PAGE and immunoblotting with HBM1 (Fig. 7). Whereas the glomeruli from control rats appeared to contain almost only one MHC isoform (MIIA), those from Thy 1.1-injected rats contained increased concentrations of two MHC isoforms, MIIA and MIIB2. These extracts again did not appear to contain an appreciable concentration of the brain-specific MHC isoform MIIB1. Quantitative analysis of the MIIB2 isoform by ELISA indicated that the level of MIIB2 began to increase at the fifth day and peaked at the tenth day and then declined gradually (Fig. 8). The level of MIIB2 in the glomeruli at the tenth day was more than four times higher than that of the control. The peak of the expression of MIIB2 thus

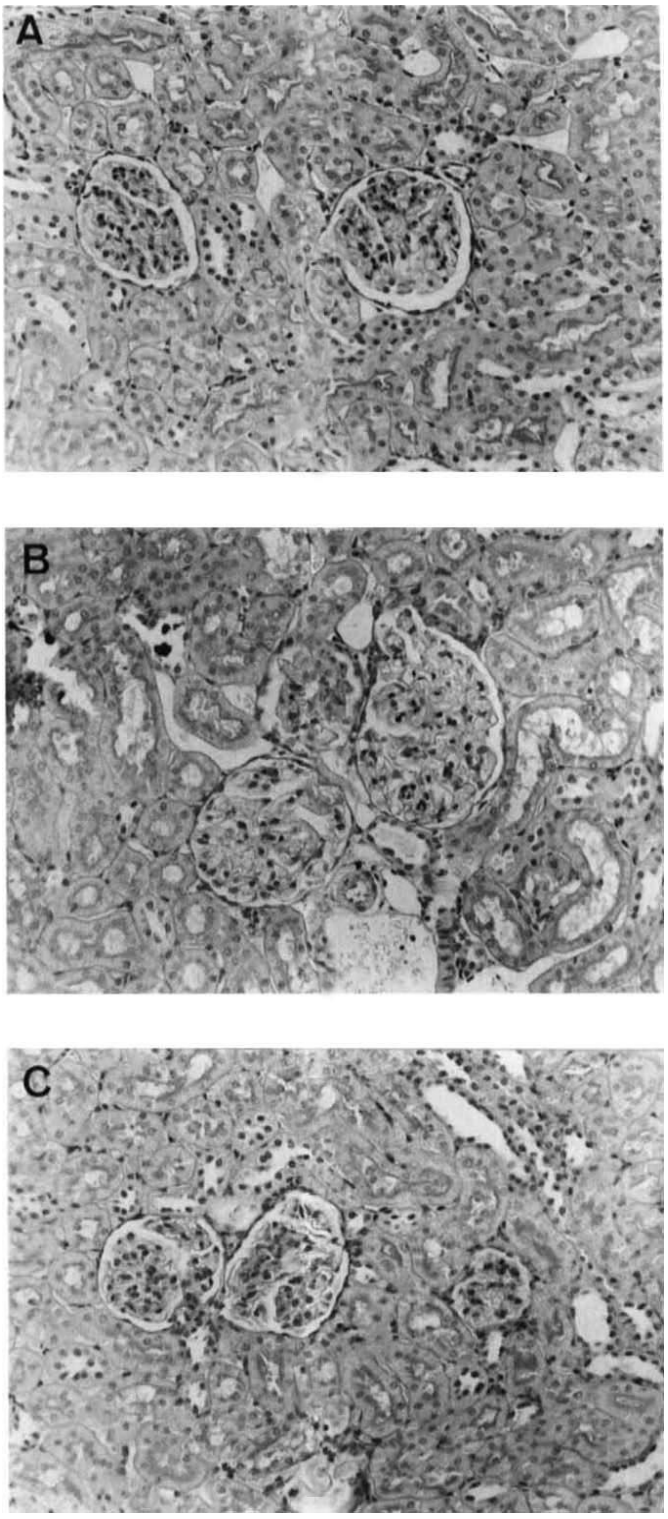

Fig. 4. *Morphological changes of rat glomeruli after injection of anti-Thy 1.1 antibody.* Rats were injected with anti-Thy 1.1 antibody and the kidneys were removed at the indicated days. The tissues specimens were stained with PAS and examined under a light microscope. Compared to control rat glomeruli (**A**, × 100), the glomerular cellularity is reduced on one day after injection of anti-Thy 1.1 (**B**, × 100). The mesangial cells are markedly proliferated at five days after injection of anti-Thy 1.1 (**C**, × 100).

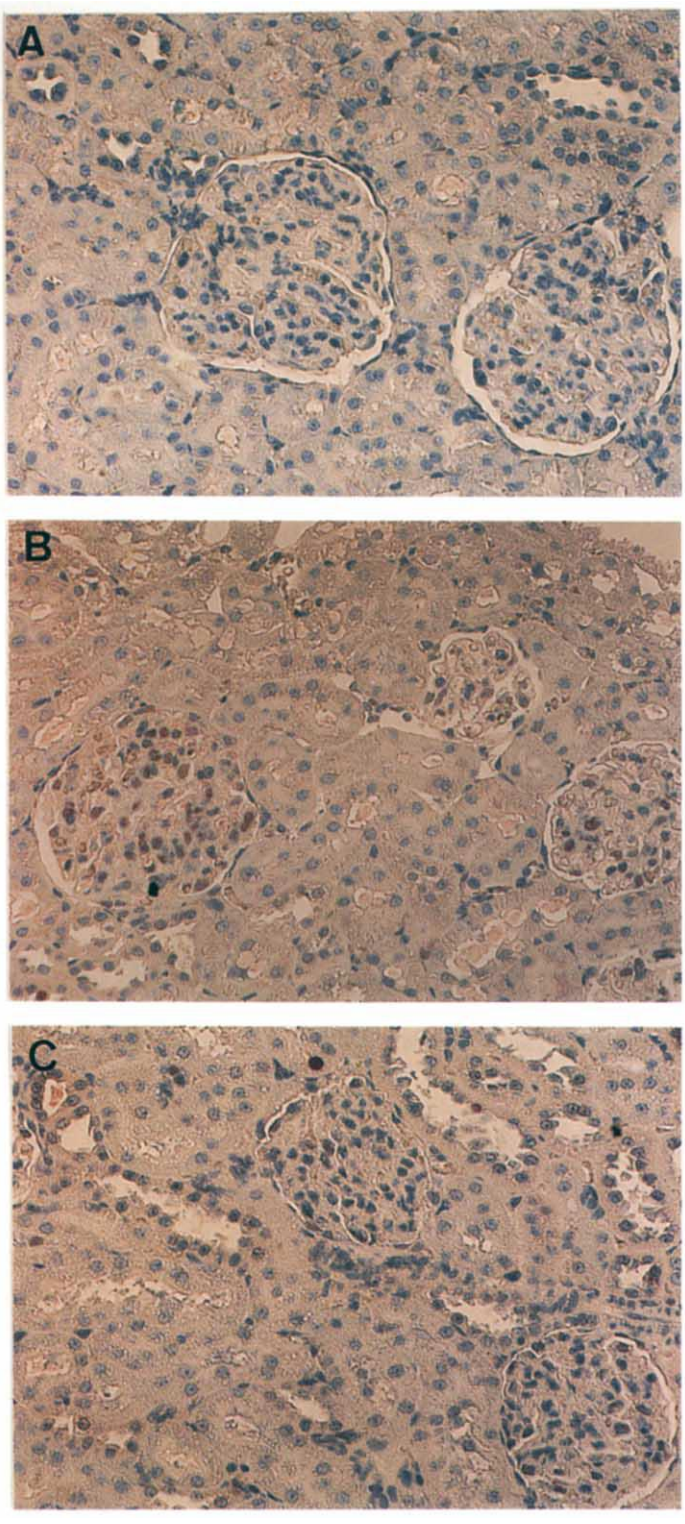

Fig. 5. *Anti-Thy 1.1-induced glomerular cell proliferation as detected by immunolabeling with PCNA.* Rats were injected with anti-Thy 1.1 antibody, and the kidneys were removed at the indicated days. The tissue specimens were stained with an anti-PCNA antibody. The glomeruli of control rats do not contain an appreciable number of the immunostains (**A**, × 100). The glomeruli of anti-Thy 1.1-injected rats contain a large number of the immunostains at five days after injection of the antibody, while a marked extent of cell proliferation is evident at day 5 after disease induction (**B**, × 100). Expression of PCNA is diminished at the 14th day (**C**, × 100).

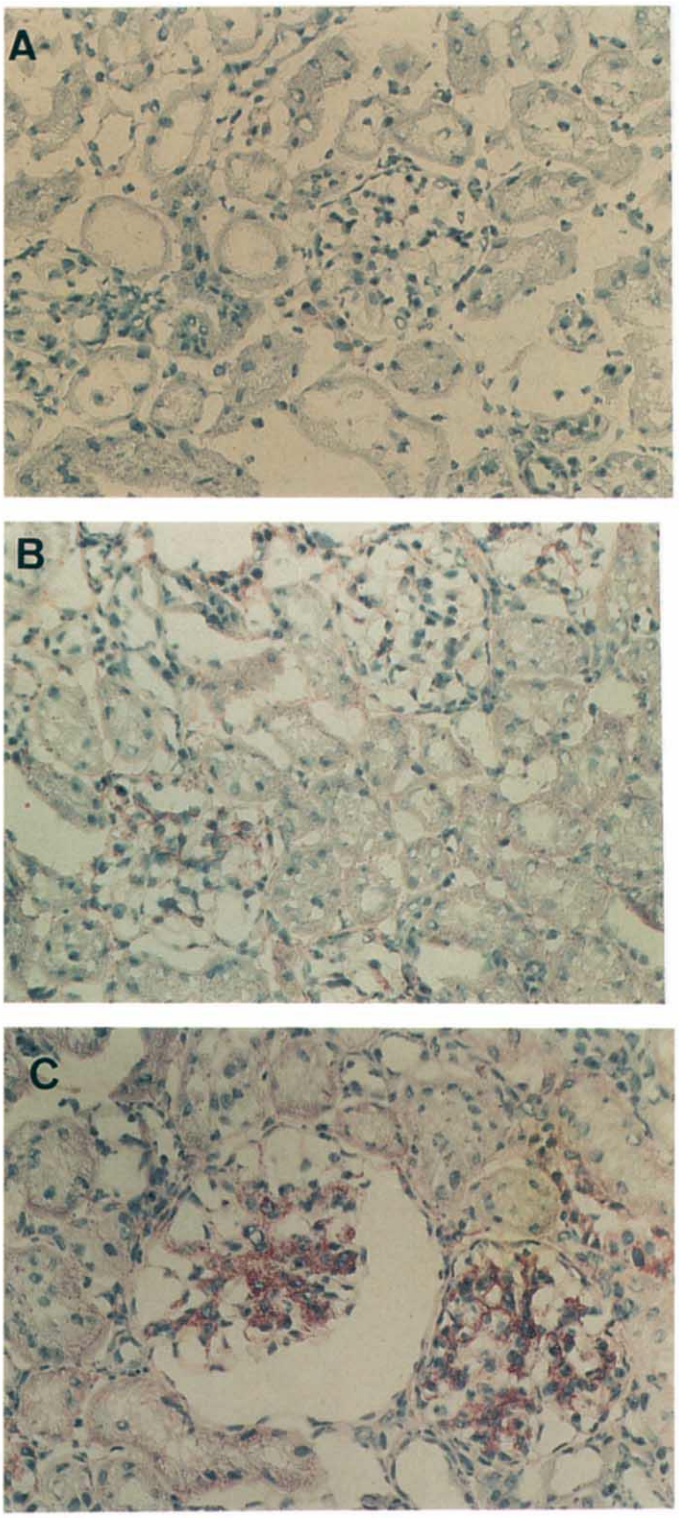

Fig. 6. *Expression of brain-type myosin in normal and diseased glomeruli.* Rats were injected with anti-Thy 1.1 antibody, and the kidneys were removed at the indicated days. The tissue specimens were stained with anti-brain-type MHC antibody HBM1. Whereas the tissue specimen from a control rat contains a significant level of the immunostains in the tubular regions, the glomerular regions do not contain an appreciable level of the immunostains (**A**, × 200). At five days after injection of anti-Thy 1.1, the numbers of the mesangial cells are increased in most of the glomeruli, while the brain-type myosin is only expressed slightly in the glomeruli (**B**, × 200). At 10 days after anti-Thy 1.1 injection, the brain-type myosin is expressed in the glomeruli in a readily detectable amount (**C**, × 200).

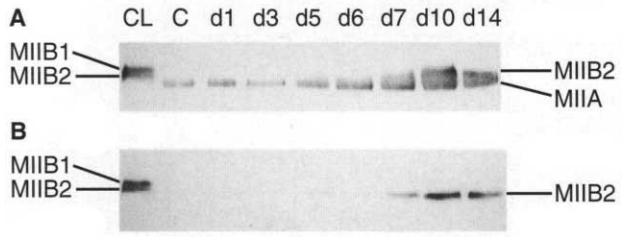

Fig. 7. *Immunoblot analysis of MHC isoforms in the glomeruli of anti-Thy 1.1 GN rats.* Rats were injected with anti-Thy 1.1 antibody, and the kidneys were removed at the indicated days (0 to 14 days) and donated for the preparation of glomeruli. Crude myosin extracts (30 μg protein/lane) prepared from the glomeruli (d0-d14) and rat cerebellum (CL) were subjected to SDS-PAGE and immunoblotting with an anti-smooth muscle, nonmuscle and brain-type MHC antibody BBM4 (**A**) or with an anti-brain-type MHC antibody HBM1 (**B**).

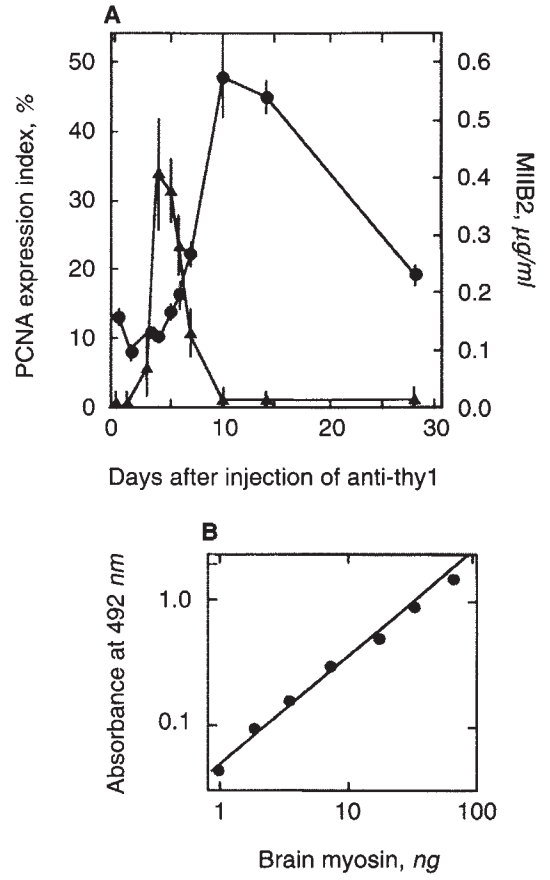

Fig. 8. *Time courses of expressions of MIIB2 isoform (●) and PCNA (▲) in the renal tissues of anti-Thy 1.1. GN rats.* (**A**) Rats were injected with anti-Thy 1.1 antibody, and the kidneys were removed at the indicated days. Crude myosin extracts were prepared from the isolated glomeruli, and the concentrations of the MIIB2 isoform in the extracts (10 μg protein) were determined by ELISA on a plate coated with anti-brain-type MHC antibody HBM1, as described previously [5, 6, 34]. Expression of PCNA in the glomeruli of anti-Thy 1.1-injected rats was determined by the histochemical and immunohistochemical procedures shown in Figures 4 and 5, respectively. PCNA-expression is expressed by the labeling index, the percentage of immunochemically stained nuclei per total nuclei. The points and bars represent the mean values ($N = 4$) and SDs, respectively. (**B**) A calibration curve for brain-type myosin with a best fit line ($r^2 = 0.994$) determined by ELISA.

appears to be delayed by approximately or more than five days from that of PCNA (Fig. 8).

Discussion

The mesangial cells have been reported to contain fairly high concentrations of both myosin and actin [27], cytoskeletal elements essential for cell motility [28], and these contractile proteins are believed to be involved in the regulation of glomerular filtration rate (GFR) by modulating the glomerular capillary filtrating surface area [29]. A previous observation that the myosin in cultured mesangial cells undergoes rapid phosphorylation in response to several agonists such as arginine vasopressin, angiotensin II, and endothelin appeared to reinforce the role of myosin in regulating the cell motility [2]. In addition to this function, the myosin is likely to play a role in cellular proliferation, since nonmuscle-type myosin has been shown to be involved in the cellular proliferation, especially in the process of cytokinesis [30–32]. A previous study has shown that the content of myosin was increased in the mesangial cells of such glomerular diseases as diabetic nephropathy [33], while it is not known with which changes of the cellular functions the increased content of myosin is related, the contractile activity or the proliferation of the cells. In the present study, we have shown that nearly all of the patients with proliferative GN contain increased concentrations of a brain-type myosin isoform in the mesangial cells and that normal rats increase the contents of the brain/embryo-type myosin isoform (MIIB2) in the mesangial cells by injecting the anti-Thy 1.1 antibody. Althogh much of the brain-type myosin isoform expression does appear to be mesangial cells in Figure 3 A and B, and Figure 6C, it is possible that some of the myosin expression may be originating from other cells within glomerulus, such as the endothelial cell or podocyte. While we were unable to determine which type of the two brain isoforms was expressed in the mesangial cells from patients with proliferative GN, MIIB1 or MIIB2, because of the limitation of the amounts of the biopy samples, the MHC expressed in these cells was likely to be mostly the MIIB2 isoform. These results indicate that the expression of the brain/embryo-type isoform is accelerated with development or/and progression of proliferative GN, although the reason for the increased expression of the isoform is not known. It is unlikely, however, that the increased expression of the myosin isoform is prerequisite to the proliferation of the mesangial cells, since (1) the expression of the isoform is induced after the expression of PCNA was almost completed (Figs. 5, 6 and 8), and (2) most of the human tissue specimens from proliferative GN did not contain a significant level of PCNA. Thus these facts are remarkably contrastive with the previous report that the expression of α-smooth muscle actin is associated with the expression of PCNA in mesangial cells in biopsy specimens from patients with GN and in rat Thy 1.1 GN [17, 18]. Therefore, it is likely that the brain/embryo-type isoform plays a role in the process different from the α-smooth muscle actin in GN. The result shown in Figure 8 also indicated that the content of the nonmuscle myosin (MIIA) was increased slightly together with the MIIB2 isoform in the rat model of GN. The increased expression of the MIIA isoform does not appear to be prerequisite to the proliferation of the mesangial cells, although this result does not exclude the

possibility that the pre-existed isoform is involved in cellular division [30–32].

Recently, we have found that regenerating rat hepatic tissues also express an increased level of the brain/embryo-type MHC isoform for a short period after partial hepatectomy [34]. The level of the MHC isoform was highest at a few days after the peak of the expression of PCNA was attained, as in the experimental GN. The MIIB2 isoform appeared to diminish with recoveries of both the size and function of the tissues, as estimated from the tissue weight and the serine dehydratase activity, respectively [34]. The result shown in Figure 8 also indicates that the anti-Thy 1.1-induced brain/embryo-type myosin gradually decreases its level toward the basal level with the recovery from the nephritis, as estimated from the morphological integrity of the tissues (not shown) [20]. On the other hand, nearly all of the renal tissues from patients with chronic proliferative GN were found to continuously contain significant levels of the isoform in the glomeruli. Whereas the reason for the successive presence of this isoform is not known, it is likely to reflect the fact that mesangial cells of GN exhibit a different phenotype from the normal cells. Although the changes in the isoform mentioned above were observed under rather specialized tissue conditions, partial hepatectomy and experimental and chronic GN, a limited number of normal adult tissues also contain varied but nearly fixed levels of the isoform. Brain tissues contain fairly high concentrations of the isoform and the renal tissues contain a small concentration of the isoform in the tubular cells. It is also evident that many fetal tissues such as liver, thymus, skeletal and cardiac muscle and arteries contain each a fairly high concentration of the isoform [10, 12]. These facts appear to suggest that the brain/embryo-type MHC isoform is involved in some process(es) that are necessary to maintain the tissues in such a specialized physiological state as in diseases and in embryo or fetus.

It has been shown recently that the development of atherosclerosis is accompanied by changes in the MHC isoforms characterized by the increase in the level of the brain/embryo-type MHC isoform MIIB2 and the decrease in the levels of the smooth muscle MHC isoforms, SM1 and SM2. These changes have been proposed to be involved in the phenotypic transformations of the muscle cell from a contractile form to a synthetic form [12, 13, 35]. On the other hand, it has also been shown that brain myosins (MIIB1 and MIIB2) exhibit a considerable magnitude of contractile activity *in vitro* in response to phosphorylation by Ca^{2+}/calmodulin-dependent myosin light chain kinase [36]. Furthermore, these myosins possess different physical and enzymatic properties from those of smooth muscle and other nonmuscle myosins. The filaments of unphosphorylated brain myosin are not dissociable in the presence of ATP or 0.3 M KCl, while those of smooth muscle and nonmuscle myosins were dissociated under the same conditions [37]. Although the mechanisms by which the brain/embryo-type myosin participates in phenotypic transformations of the mesangial cells is not known, the isoform may play a role in providing the cells with a specialized type of cellular motility occurring in GN.

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